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Fort Detrick, Maryland 21702-5012

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| 13. ABSTRACT (Maximum 200) BRCA1 is a tumor-suppressor locus on chromosome 17q21. Familial inheritance of a defective copy places a lifetime risk of breast cancer at 80%. Most of these tumors arise before the age of 50. In addition, there is an elevated risk of ovarian and testicular tumors. The mechanism of transformation is not known. In an effort to better understand the actions of this gene, I have cloned the mouse <i>Brca1</i> gene. The present proposal aims to characterize the effect of over-expression and deletion of <i>Brca1</i> in mice, and by understanding the nature of the interactions between <i>Brca1</i> and other oncogenes. The completion of these aims will provide: (1) a mouse model of <i>Brca1</i> deficiency, (2) an enhanced understanding of <i>Brca1</i> function in the regulation of mammary epithelial cell growth and differentiation, and (3) reveal important interactions between <i>Brca1</i> and other potent transforming agents in the breast, in particular with <i>c-myc</i> , and loss of p53. | | | | |
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FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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✓ ____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.


PI - Signature

10/30/98
Date

1998 Annual Report for Grant Number DAMD17-96-1-6095

Title: The Brca1 Tumor-Suppressor gene in a mouse model of breast cancer

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INTRODUCTION:

In our original application, we proposed to investigate the following specific aims:

1. Characterize of the effects of *Brcal* expression on the proliferation and differentiation of breast cancer cells of known genotype.
2. Establish mice that lack functional *Brcal* by targeted disruption.
3. Genetic complementation of *Brcal* deficient mice with strains that express oncogenes known to contribute to the development of breast cancer.

To date we have made significant progress on all three aims. In particular, we have studied the effect of overexpressing BRCA1 in mouse mammary epithelial cells and have studied the subcellular localization of the murine *Brcal* gene product (Aim 1). These result experiments were completed and summarized in last report. We have created a line of transgenic mice that overexpress human BRCA1 (MBR) (aim 1) and have created a second group of mice that overexpress an antisense construct of mouse *Brcal* (BAS) (aim 1). We have created a line of mice that carry an inactivated *Brcal* locus (BrKO) (aim 2) and have crossed the BrKO mice with mice predisposed to cancer, including ($p53^{+/-}$, $p21^{+/-}$, and MMTV-myc) (Aim 3). In the course of these experiments, we experienced several technical difficulties which hindered our progress. In particular, BrKO mice do not develop cancer and mating with cancer prone $p53^{+/-}$, $p21^{+/-}$, or MMTV-myc strains did not appear to accelerate or contribute to tumor progression of these strains.

In the last report we proposed substituting BAS (MMTV-*Brcal* antisense) mice for BrKO mice in Aim 3 and crossing them into $p53^{+/-}$ and MMTV-myc backgrounds. This experiment is now underway and we have 10-20 animals of each genotype that are being studied for tumor progression. As described below, we have had some indication that the BAS lines are tumor prone indicating that the change in emphasis was justifiable.

BODY:

Analysis of tumor formation in *Brcal* knock-out (BrKO) mice:

As described in the 1997 progress report, BrKO mice were established and have been monitored since 1996. In all respects, the phenotype of these mice appears similar to that described by other groups who have knocked out the *Brcal* gene (6, 8, 11, 12). Homozygous BrKO mice (BrKO/BrKO) display embryonic lethality at day 6-7.5 of development and thus are not useful for analysis of tumor progression. We also fail to identify tumors in heterozygous animals (BrKO $^{+/-}$), a result consistent with other groups. To date, we have analyzed over 120 mice that reached 8 mo of age or greater.

In the past year, we have focused on crossing BrKO mice with $p53$ knockout mice to obtain double heterozygotes. These mice usually develop lymphomas by 6 months of age and are otherwise similar to $p53$ knockout mice. However, we have had two of these mice develop breast tumors ($n=25$) and are in the process of breeding more animals to verify the phenotype. We have saved DNA from the tumors and will analyze the remaining *Brcal* allele for loss. We have plans to submit a request for additional funding to see if irradiation induced DNA damage could accelerate this process.

Generation of Transgenic mice expressing MMTV-*Brcal* Antisense and MMTV-BRCA1:

A) To overcome the long latency in tumor progression in BrKO $^{+/-}$, we generated several lines of transgenic mice expression a *Brcal* antisense (BAS) construct targeted to the mammary gland (Table #1). The justification and strategy for creating these mice was described in the 1997 progress report.

The new lines that were created are described in Table 1.

Table 1 Transgenic mice (new lines)

| <u>Construct/transgene</u> | <u>Name</u> | <u># of lines</u> ¹ | <u>tumor formation</u> |
|----------------------------|-------------|--------------------------------|------------------------|
| MMTV-Brcal antisense | BAS | 8 | yes (3/8 lines) |
| MMTV-BRCA1 | MBR | 1 | no |
| beta-actin-BRCA1 | | 0 | N.A. ⁴ |

¹Number of lines represents the number of founders that transmitted transgenic DNA to offspring.

⁴Not applicable, this construct appears to result in embryonic lethality.

BAS mice have been in the lab for 2.5 years. Upon dexamethasone treatment, 3 week old BAS females show evidence of mammary hyperplasia with increased numbers of branch points in the mammary tree (Figure 1). Non-treated (dexamethasone free) females develop mammary adenocarcinomas with long latency (6-12 months (Figure 2). The incidence is low (4/20 mice > 8mo of age). We are currently mating BAS mice into the p53 null background, following the same procedure outlined for the BrKO mice in the original application. The bigenic BAS/p53 colony is still young, but we have observed 4 mammary tumors thus far and are awaiting additional results as the colony ages. The strategy of targeting Brcal antisense appears to be a more effective means of eliminating Brcal expression than waiting for allelic loss at the endogenous Brcal locus. It therefore is a very promising alternative for studying BRCA1 induced tumors.



Figure 1 Expression of Brcal antisense in mammary epithelial cells leads to increased branching and hyperplasia. Panel A shows a whole mount preparation a wild-type mammary gland from a 4 week old mouse treated for 7 days with dexamethasone. Panel B shows a similar preparation from a BAS transgenic littermate. Arrows identify branch points which are much more prevalent in the BAS animal.

We have also generated a number of BASxMMTV-myc mice. At present the females have not developed tumors (average age 3 months) but they are being monitored closely.

Strategy:

The strategy is thus to continue analyzing BrKOxp53 and BAS mice for tumor progression. We are establishing cell lines from the BAS mice in the hopes of demonstrating reduced Brcal protein expression. We will analyzed all tumors for LOH at the BRCA1 and p53 loci.



Figure 2 Expression of Brcal antisense in mammary epithelial cells leads to the development of mammary adenocarcinomas. The Panel shows a 5 um histological section of mammary tissue obtained from an BAS female. The right side of the panel shows non transformed mammary tissue including ducts (arrows) and adipocytes (a). The left side of the panel shows a well developed adenocarcinoma.

Conclusions:

At the end of year 2, we have made progress on all 3 Aims. We have shown that mouse Brcal is a nuclear protein that blocks cell proliferation when overexpressed. We have also shown that Brcal is an essential gene. Loss of the gene in BrKO mice results in early embryonic lethality. Heterozygous BrKO animals are healthy and do not appear to show increased susceptibility to breast cancers, or to any other disease states. While the lack of disease in BrKO mice has been disappointing, the Brcal antisense (BAS) approach appears to be working. Specifically, we appear to be able to reduce Brcal protein levels to the point where we can observe increased proliferation (hyperplasias) without inducing cellular lethality. We will continue characterizing these mice. To date 3 out of 8 BAS lines have developed at least one mammary tumor and we are particularly focused on line G which appears particularly cancer prone. We will continue analyzing dexamethasone responsiveness, and sensitivity to DNA damage mediated by ionizing radiation. We will present these results in the final report. As stated, we are in the proceeding with complementation experiments originally proposed in Aim 3 with the BAS transgenics instead of the BrKO mice.

REFERENCES:

1. Chen, Y., C. F. Chen, D. J. Riley, D. C. Allred, P. L. Chen, D. Von Hoff, C. K. Osborne, and W. H. Lee. 1995. Aberrant subcellular localization of BRCA1 in breast cancer. *Science*. 270:789-791.
2. Chen, Y., A. A. Farmer, C. F. Chen, D. C. Jones, P. L. Chen, and W. H. Lee. 1996. BRCA1 is a 220-kDa nuclear phosphoprotein that is expressed and phosphorylated in a cell cycle-dependent manner. *Cancer Res*. 56:3168-3172.
3. Deng, C., P. Zhang, J. W. Harper, S. J. Elledge, and P. Leder. 1995. Mice lacking p21^{CIP1/WAF1} undergo normal development, but are defective in G1 checkpoint control. *Cell*. 82:675-684.
4. Donehower, L. A., L. Godley, C. Aldaz, R. Pyle, Y. Shi, D. Pinkel, T. Gray, A. Bradley, and H. E. Varmus. 1995. Deficiency of p53 accelerates mammary tumorigenesis in wnt-1 transgenic mice and promotes chromosomal instability. *Genes Devel*. 9:882-895.
5. Futreal, P. A., Q. Liu, D. Shattuck-Eidens, C. Cochran, K. Harshman, S. Tavtigian, L. Bennett, A. Haugenstrano, J. Swensen, Y. Miki, K. Eddington, M. McClure, C. Frye, J. Weaverfeldhaus, W. Ding, Z. Gholami, P. Soderkvist, L. Terry, S. Jhanwar, A. Berchuck, J. Iglehart, J. Marks, D. G. Ballinger, J. C. Barrett, M. H. Skolnick, and e. al. 1994. BRCA1 mutations in primary breast and ovarian carcinomas. *Science*. 266:120-122.

6. Gowen, L. C., B. L. Johnson, A. M. Latour, K. K. Sulik, and B. H. Koller. 1996. Brca1 deficiency results in early embryonic lethality characterized by neuroepithelial abnormalities. *Nat Genet.* 12:191-194.
7. Hakem, R., J. L. de la Pompa, A. Elia, J. Potter, and T. W. Mak. 1997. Partial rescue of Brca1 (5-6) early embryonic lethality by p53 or p21 null mutation. *Nat Genet.* 16:298-302.
8. Hakem, R., J. L. de la Pompa, C. Sirard, R. Mo, M. Woo, A. Hakem, A. P. Wakeham, J., A. Reitmair, F. Billia, E. Firpo, C. C. Hui, J. Roberts, J. Rossant, and T. W. Mak. 1996. The tumor suppressor gene Brca1 is required for embryonic cellular proliferation in the mouse. *Cell.* 85:1009-1023.
9. Jin, Y., X. L. Xu, M. C. W. Yang, F. Wei, T. C. Ayi, A. M. Bowcock, and R. Baer. 1997. Cell cycle-dependent colocalization of BARD1 and BRCA1 proteins in discrete nuclear domains. *Proc Natl Acad Sci U S A.* 94:12075-12080.
10. Lane, T. F., C. Deng, A. Elson, M. S. Lyu, C. A. Kozak, and P. Leder. 1995. Expression of Brca1 is Associated with Terminal Differentiation of Ectodermally- and Mesodermally-derived Tissues in Mice. *Genes Dev.* 9:2712-2722.
11. Liu, C. Y., A. Flesken-Nikitin, S. Li, Y. Zeng, and W. H. Lee. 1996. Inactivation of the mouse Brca1 gene leads to failure in the morphogenesis of the egg cylinder in early postimplantation development. *Genes Dev.* 10:1835-1843.
12. Ludwig, T., D. Chapman, V. Papaioannou, and A. Efstratiadis. 1997. Targeted mutations of breast cancer susceptibility gene homologs in mice: lethal phenotypes of Brca1, Brca2, Brca1/Brca2, Brca1/p53, and Brca2/p53 nullizygous embryos. *Genes Dev.* 11:1226-1241.
13. Miki, Y., J. Swensen, D. Shattuck-Eidens, P. Futreal, K. Harshman, S. Tavtigian, Q. Y. Liu, C. Cochran, L. M. Bennett, W. Ding, R. Bell, J. Rosenthal, C. Hussey, T. Tran, M. McClure, C. Frye, T. Hattier, R. Phelps, A. Haugenstrano, H. Katcher, K. Yakumo, Z. Gholami, D. Shaffer, S. Stone, S. Bayer, and M. H. Skolnick. 1994. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science.* 266:66-71.
14. Muller, W. J., E. Sinn, P. K. Pattengale, R. Wallace, and P. Leder. 1988. Single-step induction of mammary adenocarcinoma in transgenic mice bearing the activated c-neu oncogene. *Cell.* 54:105-115.
15. Scully, R., J. Chen, R. L. Ochs, K. Keegan, M. Hoekstra, J. Feunteun, and D. M. Livingston. 1997. Dynamic changes of BRCA1 subnuclear location and phosphorylation state are initiated by DNA damage. *Cell.* 90:425-435.
16. Scully, R., J. Chen, A. Plug, Y. Xiao, D. Weaver, J. Feunteun, T. Ashley, and D. Livingston. 1997. Association of BRCA1 with Rad51 in mitotic and meiotic cells. *Cell.* 88:265-275.
17. Thomas, J. E., M. Smith, B. Rubinfel, M. Gutowski, R. P. Beckmann, and P. Polakis. 1996. Subcellular localization and analysis of apparent 180-kDa and 220-kDa proteins of the breast cancer susceptibility gene, BRCA1. *Biol Chem.* 271:28630-28635.
18. Thompson, M. E., R. A. Jensen, P. S. Obermiller, D. L. Page, and J. T. Holt. 1995. Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. *Nature Genetics.* 9:444-450.
19. Wang, H., N. Shao, Q. M. Ding, J. Cui, E. S. Reddy, and V. N. Rao. 1997. BRCA1 proteins are transported to the nucleus in the absence of serum and splice variants BRCA1a, BRCA1b are tyrosine phosphoproteins that associate with E2F, cyclins and cyclin dependent kinases. *Oncogene.* 15:143-157.
20. Wilson, C. A., M. N. Payton, G. S. Elliott, F. W. Buaas, E. E. Cajulis, D. Grosshans, L. Ramos, D. M. Reese, D. J. Slamon, and F. J. Calzone. 1997. Differential subcellular localization, expression and biological toxicity of BRCA1 and the splice variant BRCA1-delta11b. *Oncogene.* 9:1-16.

U.S. Army Medical Research and Materiel Command Animal Use Report

Facility Name: UCLA, Div. of Laboratory Medicine Principal Investigator: Timothy F. Lane
 Address: Box 951740, CHS-27-139
10833 Le Conte Ave, Principal Investigator: Timothy F. Lane
Los Angeles CA 90095-1740 (Typed/Printed Name)

Contract Number: DAMD17-96-1-6095

This Report is for Fiscal Year (01 October - 30 September): 1998

AAALAC* Accreditation Status (circle one): (Full) Provisional Not Accredited

Date of Last USDA Inspection: June 3 1998 USDA Registration Number: 93-R-0446

| Definitions of Column Headings on Back of Form | | | | | |
|--|--|---|--|--|--|
| A. Animal | B. Number of animals purchased, bred, or housed but not yet used | C. Number of animals used involving no pain or distress | D. Number of animals used in which appropriate anesthetic, analgesic, or tranquilizing drugs were used to alleviate pain | E. Number of animals used in which pain or distress was not alleviated | F. Total Number of Animals (Columns C+D+E) |
| Dogs | | | | | |
| Cats | | | | | |
| Guinea Pigs | | | | | |
| Hamsters | | | | | |
| Rabbits | | | | | |
| Non-human Primates | | | | | |
| Sheep | | | | | |
| Pigs | | | | | |
| Goats | | | | | |
| Horses | | | | | |
| Mice | 125 125 | 125 | | | 125 |
| Rats | | | | | |
| Fish | | | | | |
| List Others: | | | | | |
| | | | | | |
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*AAALAC - Association for the Assessment and Accreditation of Laboratory Animal Care



United States Department of Agriculture
Animal and Plant Health Inspection Service
Animal Care

INSPECTION REPORT

University of California- Los Angeles
Department of Laboratory Animal
Medicine
630 Circle Drive
Los Angeles, CA

Site 01
Main Vivarium

93-R-0044 AG
05-22,27-98
0930
Routine

NARRATIVE

Animal Inventory- 18 Dogs

- 14 Cats
- 35 Guinea pigs
- 11 Hamsters
- 208 Rabbits
- 21 Chinchillas
- 38 Deer mice
- 25 Non-human primates(11 baboons, 2 macaques, 12 squirrel monkeys)
- 46 Domestic swine

CATEGORY III: Non-compliant item(s) identified this inspection

Section 3.2(d), 3.26(d), 3.51(d), 3.75(c)- Interior Surfaces - The following areas were noted with unsealed, porous surfaces which can not be cleaned and sanitized as required-

- peeling paint, pitted floors, wallboards separating from walls (porous wallboard and concrete exposed) - rooms housing non-human primates
- tile missing from wall (porous wallboard exposed)- guinea pig room
- brick floors with peeling sealant- rabbit room
- peeling paint on walls- rooms housing dogs
- peeling paint, pitted floors (porous wallboard and concrete)- cagewashing and bedding storage rooms

All above to be corrected by: 8-3-98

Note: All above reported to facility maintenance department prior to this inspection.

Prepared By: Kathleen M. Garland, DVM, VMD
Title: Kathleen M. Garland, Veterinary Medical Officer, USDA, APHIS, Animal Care

Date: 6-3-98
LARIS ID NO. 5006

Copy Received By: John B. Pickett
Title: Director, IACUC

Date: 6/3/98

05-22,27-98

Section 3.11(c), 3.31(b), 3.58(c), 3.84(c), 3.131(c) - Housekeeping - Floor drain cover missing from dog recovery room - ~~To be corrected by:~~ Corrected at time of inspection
- Florescent light not functioning properly in surgery room - ~~To be corrected by:~~ Corrected at time of inspection
- Wall ventilation duct cover noted with build-up of dirt and dust in surgery room - Corrected at time of inspection

Section 3.129(b) - Feeding - Pigs are fed by placing feed in rubber tubs- some of these
Section 2.33(b) - Veterinary Care - rubber tubs noted with areas missing (chewed off) and areas that have been worn and chewed so that fibers are exposed- these tubs can not be kept cleaned and sanitized as required. Also, there is a concern that the animals may be ingesting pieces or fibers of rubber which may result in impaction or other injury to the animals.
~~To be corrected by~~ Corrected at time of inspection

Section 2.33(b) - Veterinary Care - Expired medications noted mixed with current dated drugs in the surgery and treatment rooms- the following were observed:
Depoprovera- expired 2-97
Oxytetracycline- expired 7-97
Heparin- expired April 1, 1998

All above were removed at time of inspection - corrected at time of inspection

Section 3.81- Environmental Enhancement - This facility houses baboons, squirrel monkeys, and macaques, but does not account for, or document, species differences in the written program of environmental enhancement/ behavioral enrichment. Although it is documented in the written plan that each non-human primate room is provided with intercom-supplied music throughout the day, it was not noted at time of inspection. To be corrected by: 7-3-98

Note: Development of environmental enhancement plans, protocol literature searches and protocol / program reviews were discussed.

End of Report

Prepared By: Kathleen M. Garland, DVM, VMD
Title: Kathleen M. Garland, Veterinary Medical Officer, USDA, APHIS, Animal Care

Date: 6-3-98
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